Report for 2003MS19B: Chemical Mixtures: Consequences of WNV Eradication on Water Quality

There are no reported publications resulting from this project.

RESEARCH PROPOSAL

(1) TITLE: Chemical Mixtures: Consequences of WNV Eradication on Water Quality

(2) Focus Categories: SED, TS, WQL

(3) Keywords: Ecosystems, Mixtures, Pesticides, Residues, Sediments, Toxic Substances, Water Quality

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(7) Principal Investigator, University and City: Marc Slattery, University of Mississippi, University, MS

(8) Congressional District No: District No.1

(9) Water Problem, Need for Research:

Recent outbreaks of West Nile Virus (WNV) throughout the United States, and particularly in the Mississippi Valley States, have spurred plans to control the vector (= *Culex* mosquito). A probable phase in each plan requires using chemical agents that affect either adult or larval vector life stages. Chemical agents commonly used to control mosquito vectors are non-species specific pesticides that will potentially interact with non-target aquatic organisms. These compounds enter the aquatic environment via direct or indirect routes eventually becoming part of water and sediment matrices. Most of the WNV vector control compounds are hydrophobic. Upon entering the aquatic environment they readily partition from surface waters onto particulate organic matter in the water column or directly onto the sediment. Within aquatic matrices through direct contact, respiration or indirect ingestion non-target organisms are exposed to vector control compounds individually or as mixtures with persistent or transient anthropogenic compounds such as regional crop pesticides and metals. Individually or as mixtures, acting additively or synergistically, these compounds can potentially affect adult and juvenile life stages of non-target organisms. At the present time, there is limited knowledge regarding effects of WNV vector control compounds in mixtures. Evaluating water quality and aquatic habitat are critical to an overall assessment of vector eradication programs.

This proposal directly addresses Mississippi Water Research and South Atlantic-Gulf Region priorities related to water quality, particularly with respect to needs addressing protection of water and sediment from environmental degradation.

(10) Expected Results, Benefits, Information:

Water and sediment quality in aquatic environments are essential indicators of overall success of WNV vector control programs. Aquatic matrices are complex mixtures of natural and anthropogenic compounds. Our proposed research encompasses both individual compound and mixture exposure studies in both water and sediment matrices. We will be able to assess

toxicological effects not predicted by individual compound toxicity studies. Our pre-stress exposure experiments will allow us to evaluate model organisms' responses to vector control compounds after pre-exposure to commonly occurring persistent anthropogenic compounds. We will compare critical body residue values determined from controlled laboratory studies to tissue residues from exposed organisms collected from areas during vector control application. By comparing residue levels we can more accurately evaluate risk to aquatic organisms during vector control application periods. During periods of environmental application of vector control compounds we will evaluate water and sediment samples for mixture concentrations of vector control and commonly occurring anthropogenic compounds. By mimicking environmental mixture concentrations in controlled exposure studies we can assess "real-world" chemical mixture toxicological effects in model organisms commonly found in water column and sediment habitats.

In summary, the proposed research utilizes a novel approach to address the issue of chemical mixture toxicity. The model chemicals were selected to assess the influence of WNV vector eradication compound effects in conjunction with two persistent and interacting compounds in the environment that have the potential for occurrence as mixtures. Results of the proposed investigation will contribute to our currently limited understanding of chemical-chemical interactions. Accordingly, this project is directly applicable to Mississippi and the South Atlantic-Gulf because of the importance of accurately assessing ecological risk.

(11) Nature, scope, and objectives of the research:

The rapid spread of WNV throughout the United States in 2002 resulted in 3231 laboratory-verified infections and 176 deaths (as of October 21st 2002); cases in Mississippi rank within the top 5 nationwide with 178 infections and 9 deaths. Public outcry resulted in hasty plans for eradication of the *Culex* spp. mosquito vectors via insecticide spraying; *these plans often were developed locally and without much consideration to environmental and/or economic consequences.* This proposal directly addresses Mississippi Water Research and South Atlantic-Gulf Region priorities related to water quality, particularly with respect to needs addressing protection of water and sediment from environmental degradation. The following is our three–year approach for assessing impacts of WNV vector control compounds on the aquatic environment.

Phase I - Single Chemical Exposures/Insecticide, Analytical Method Development. *H. azteca*, and *D. magna*, will be exposed to single chemicals to determine concentration threshold values at which adverse toxicological effects occur. In particular, we will focus on those compounds for which this information is not reported in the literature (see Table 1 and 2). Long-term exposures will be conducted to evaluate the effects of individual chemicals on survival, growth and reproduction. Estimates of no observed effect concentrations (NOECs) and EC_{50}'s for individual compounds will be calculated. Whole body residue concentrations and toxicological effect levels will be used to calculate bioconcentration factors and critical body residues for each compound in both *H. azteca* and *D. Magna*. Targeted WNV vector eradication compounds will be spiked into water and sediment for liquid:liquid or liquid:solid extractions/recovery experiments. The extracts will be separated and quantified using LC-MS analysis, and the methods refined for future use in field matrices.

Phase II - Multiple Chemical Exposures, Pre-exposure Stress Responses. Mixture toxicity experiments evaluating binary and three ways chemical-chemical interactions of select vector control compounds (see Table 2) with two anthropogenic compounds, chlorpyrifos and methylmercury (see Table 1), will be conducted during the second year of our investigation. Each vector control/anthropogenic compound mixture study will consist of three binary and one three ways combination at selected concentrations and ratios. Additionally each mixture study will include single chemical concentrations and a control group. Fifteen replicates of each exposure level will be necessary to adequately meet the requirements of the statistical model. Juvenile *H. azteca* and adult *D. magna* will be exposed ten days and seven days, respectively, with survival, growth and reproduction as toxicological endpoints. We will also conduct these experiments in the manner of pre-exposure to a binary combination of chlorpyrifos and methylmercury, followed by addition of a vector control compound to assess the effects of pre-exposure stress on our model organisms' survival, growth, and reproduction.

Phase III - Assessment of Bioaccumulation/Field concentrations. During the third year of investigation, concentrations of the WNV vector eradication compounds, chlorpyrifos and methylmercury in water and sediment from natural waterways throughout Mississippi will be assessed using our LC-MS methodology. Also, whole body residues of the compounds mentioned above will be assessed in field collected *H. azteca* and *D. magna* and respective bioconcentration factors calculated. Additional ten-day and seven-day experiments will be conducted using spiked formulated sediment or water at environmentally relevant concentrations. Bioconcentration of the chemical mixtures will be determined from body residue analysis and chemical concentrations in the water and sediment. Environmentally relevant critical body residues will be derived through correlation of toxicity data (if any) and bioconcentration data.

(12)Methods, procedures, and facilities:

General Methods

Model Compounds. Two model compounds (chlorpyrifos and methylmercury, Table 1) representing environmentally relevant chemical contaminants will be used to assess chemical mixture interactions with the WNV vector eradication compounds (Tables 2). These chemicals were selected due to their persistence, mode of action, and occurrence at concentrations capable of producing adverse toxicological effects. In addition, during a previous study of chemical mixtures (Benson, Block, Steevens, Allgood & Slattery, 2000, Figure 2) we noted that these two compounds provided the most important additive effects (see below: Progress on Work to Date).

Chlorpyrifos. Chlorpyrifos, a model organophosphate, is widely used in the United States with more than 14.4 million pounds applied to cropland each year (USGS, 1997). Chlorpyrifos can enter the environment by volatilization and run-off after application. Following a rainfall event, streams near agricultural fields in northern Mississippi have been shown to receive concentrations of greater than 2.0 ppb chlorpyrifos in runoff 160 days after pesticide application (Smith *et al.*, 1994). Due to the low solubility (1.4 mg/L) and hydrophobic nature (Log Kow 3.31-5.27), chlorpyrifos rapidly partitions from the water and adsorbs to sediment particles (Montgomery, 1993). In the sediment, chlorpyrifos has a long half-life (60-100 days)

making exposure to aquatic benthic organisms possible (Tomlin, 1994). Chlorpyrifos exerts its toxicity by inhibiting acetylcholinesterase, an important enzyme that modulates the concentration of the neurotransmitter acetylcholine.

Methylmercury. Approximately 4,500 metric tons of mercury is released into the environment each year by human activities such as combustion of fossil fuels and other industrial releases (Lindquist *et al.*, 1991). Anthropogenic sources account for nearly 30-60% of the total annual influx of mercury to the atmosphere (Benoit *et al*., 1994). Global mercury loading trends indicate that atmospheric concentrations are increasing annually by greater than 1 percent (Slemr and Langer, 1992). In Mississippi, concern over mercury in the environment has increased as a result of increased mercury concentrations in fish tissue samples from the Sunflower and Yazoo River Basins and Enid Lake drainage (Bass, personal communication). Methylmercury is persistent in sediments and has been shown to bioaccumulate and biomagnify in fish and invertebrates as reviewed by Suedel *et al.* (1994). Methyl mercury can accumulate by way of an L amino acid transporter and exerts its toxicity by depleting cellular stores of the antioxidant glutathione or by inducing oxidative stress (Lund *et al.*, 1991; Sorenson, 1991; Mokrzan *et al.*, 1995).

Table 1. Physical-Chemical Properties of Model Compounds

Compound	Formula and Molecular Wt.	Solubility Water (@ 25° C)	log K_{ow}	log K_{oc}	Mode of Action	Stability in Water Soil
Model Cmpds						
Chlorpyrifos [a]	$C_9H_{11}Cl_3NO_3PS$ 350.6	1.4 mg/L	4.70	3.78	Non-systemic Cholinesterase Inhibitor	Low-Mod Persistence Moderate Persistence
Methylmercury [b]	CH_3Hg 215.6				Many physiological systems effected	High Persistence

Sources: EXTOXNET, 1996. Kow = octanol/water partitioning coefficient. Koc = organic carbon partitioning coefficient.

Methoprene. As part of WNV control plans in 2000, officials in New York state planned to use 135,000 pounds of methoprene containing briquets, while Westchester County planned to use as much as 80,000 pounds (Dee, 2000). When applied to larval stages methoprene mimics insect growth regulation hormone preventing metamorphosis to adults (ETOXNET, 1995). Methods of application include ground and aerial spraying, granular, mineral block/pellet and slow release briquette. Due to its low water solubility, 1.4 mg/L, and lipophilic nature, log K_{ow}, 5.21 (Table 2), methoprene readily partitions from surface water into sediments and particulate organic matter in the water column. Methoprene is acutely toxic to some freshwater and estuarine invertebrates and can bioaccumulate in fish and crustaceans (bioconcentration factor: bluegill sunfish is 457 and in crayfish 75, which is 66 times ambient water concentrations. U.S. EPA, 1982). In fresh and salt water methoprene has a half-live of between 10 and 35 days. In soil the reported half-life is 10 days (ETOXNET, 1995).

Table 2. Physical-Chemical Properties of Mosquitocides Targeted for WNV Vector Eradication

Compound	Formula and Molecular Wt.	Solubility Water (@ 25° C)	log K_{ow}	log K_{oc}	Mode of Action	Stability in Water Soil
Larvicides						
Temephos	$C_{16}H_{20}O_6P_2S_3$ 466.5	0.03 mg/L	4.91	5.0 (est.)	Cholinesterase Inhibitor	Low Persistence Low-Mod Persistence
Methoprene	$C_{19}H_{34}O_3$ 310.5	1.4 mg/L	5.21		Mimics Insect Growth Regulator	Degrades Rapidly Low Persistence
Diflubenzuron	$C_{14}H_9ClF_2N_2O_2$ 310.7	0.08 mg/L (pH 5.5, 20° C)	3.89 (log P)	4.00	Chitin Synthesis Inhibitor	Low-Mod Persistence Low Persistence
Adulticide						
Malathion	$C_{10}H_{19}O_6PS_2$ 330.3	145 mg/L	2.75	3.26	Non-systemic Cholinesterase Inhibitor	Low-Mod Persistence Low Persistence
Naled	$C_4H_7Br_2Cl_2O_4P$ 380.8	practically insoluble		2.26	Non-systemic Cholinesterase Inhibitor	Rapidly hydrolyzed Rapidly Degrades
Permethrin	$C_{21}H_{20}Cl_2O_3$ 391.3	0.2 mg/L (20° C)	6.10 (log P)	5.00	Non-systemic Insecticide	Low Persistence Low-Mod Persistence
Resmethrin	$C_{22}H_{26}O_3$ 338.4	37.9 ug/L	5.43	5.00	Non-systemic Insecticide	Low-Mod Persistence Low-Mod Persistence

Sources: Crop Protection Publications, 1994, and EXTOXNET (http://ace.ace.orst.edu/info/extoxnet/, 10/23/02).
K_{ow} = octanol/water partitionin g coefficient. K_{oc} = organic carbon partitioning coefficient.

Model Organisms. *Hyalella azteca* (Class Crustacea, Order Amphipoda) is a benthic amphipod found in fresh and estuarine waters of North and South America. *H. azteca* is exposed to environmental xenobiotics because it primarily feeds and lives in the upper layers of sediment where the concentration of contaminants is often the greatest. Physiologically, amphipods are similar to crustaceans such as crabs, crawfish, and shrimp (Gardiner, 1972; Pennak, 1989). *H. azteca* is a sentinel testing species for benthic aquatic invertebrates, which are a major food source for commercially important fishes. *H. azteca* has been used to assess bioaccumulation of metals and toxicity of sediments (Borgmann et al., 1991; Ingersoll *et al.*, 1994; Canfield *et al.*, 1994). Furthermore, *H. azteca* has been endorsed as an aquatic invertebrate testing organism for evaluating toxicity as demonstrated by the U.S. EPA standard guidelines for sediment testing (U.S. EPA, 1994b). *H. azteca* obtained from the U.S. Geological Service, National Biological Service are presently cultured in a flow-through aquarium system in the aquatic toxicology research facility of the School of Pharmacy at the University of Mississippi.

Daphnia magna (Class Crustacea, Order Anomopoda) is a cladoceran found in the water column of fresh waters of North America. *D. magna* is exposed to environmental xenobiotics within the water column and from bioaccumulated toxins within its phytoplankton prey; it is an important trophic link between primary producers and macro-predators. Physiologically, cladocerans are similar to crustaceans such as crabs, crawfish, and shrimp (Gardiner, 1972; Pennak, 1989). *D. magna* is a sentinel testing species for aquatic invertebrates, which are a major food source for commercially important fishes. Furthermore, *D. magna* has been endorsed as an aquatic invertebrate testing organism for evaluating toxicity as demonstrated by the American Society for Testing and Materials standard guidelines for acute testing (ASTM 1980: E729-80). *D.*

magna is commercially-available, and have been cultured in a flow-through aquarium system in the aquatic toxicology research facility of the School of Pharmacy at the University of Mississippi.

Toxicological Evaluation. Individual compound and binary and three ways mixtures will be exposed to H. azteca and D. magna. Endpoints evaluated will be survival, growth, reproduction and bioaccumulation (tissue residues). From tissue residues obtained from bioaccumulation experiments and toxicological data from individual compound and mixture studies critical body residues will be calculated.

Table 3. Ecotoxicology Information Relevant to Proposed Research

Compound	Percent Active Ingredient	*Daphnia* [b] µg/L		*Hyalella azteca* µg/L	
		LC_{50}	EC_{50}	LC_{50}	EC_{50}
Larvicides					
Temephos (Abate) [a,c]	5 - 43	0.011 - 0.54	----	----	----
Methoprene (Altosid) [a]	----	----	89 [d] - 360 [i]	----	----
Diflubenzuron [e]	25 - 97.6	----	7.1 - 16	----	----
Adulticide					
Malathion [a,f]	57 - 95	----	1 - 2.2	----	----
Naled [g]	58 - 91.6	----	0.3 - 1.55	----	----
Permethrin [i]	----	----	0.60	----	----
Resmethrin [i]	----	----	3.7	----	----
Model Compound					
Chlorpyrifos [a,h]	25.6 - 97.7	0.10 -115	----	0.119 - 0.219 [j]	----
Methylmercury [a]	97.0 [j] (CH_3HgCl)	----	----	3.8 - 23.5 [j]	3.2 - 10 [k]

a: Bioaccumulates or potential to bioaccumulate in aquatic organisms. b: Daphnia species not stated.
c-h: EPA's website, see references. i: Crop Protection Publications, 1994. j: Benson et al, 2000.
k: Borgmann et al., 1993

Water Exposures. Single chemical water-only experiments for both *H. azteca* and *D. magna* will be conducted to characterize the effect-concentration of the model chemicals and the WNV vector eradication insecticides that have not been previously characterized (Benson, Block, Steevens, Allgood & Slattery, 2000, and see Table 3). Acute toxicity, 48-hr, tests will be carried out using a modification of methods outlined by U.S. EPA (1991). Water quality parameters monitored include: dissolved oxygen, pH, ammonia, hardness, alkalinity, and salinity. Exposure chambers, in replicate, consist of a 30 mL beaker containing 25 mL of water/toxicant, nitex substrate, and one adult organism. At termination of the test surviving organisms are counted. *D. magna* reproduction will be evaluated using a 7-day exposure to mixtures of model compounds and vector control compounds following standard methods outlined by U.S. EPA (1989). Static water renewals will occur daily and before transferring adults to the new media one drop of food will be added to exposure chambers. After about the third day *D. magna* should start producing offspring, each day thereafter the number off offspring produced, number of broods and number of live and dead organisms will be recorded. In order to get enough tissue

to analyze for bioaccumulation large numbers of *H. azteca* and *D. magna* will be exposed for 96-hours to mixtures of model compounds at concentrations that effects have been observed. Water quality and feeding will be conducted daily. Exposure chambers consist of a 1000 mL glass beaker containing 100 adult organisms, nitex substrate, and 800 mL of test water. At termination of the exposures surviving organisms and offspring will be separated, counted and placed in vials to be stored at -80°C until tissue residues of test compounds can be quantified.

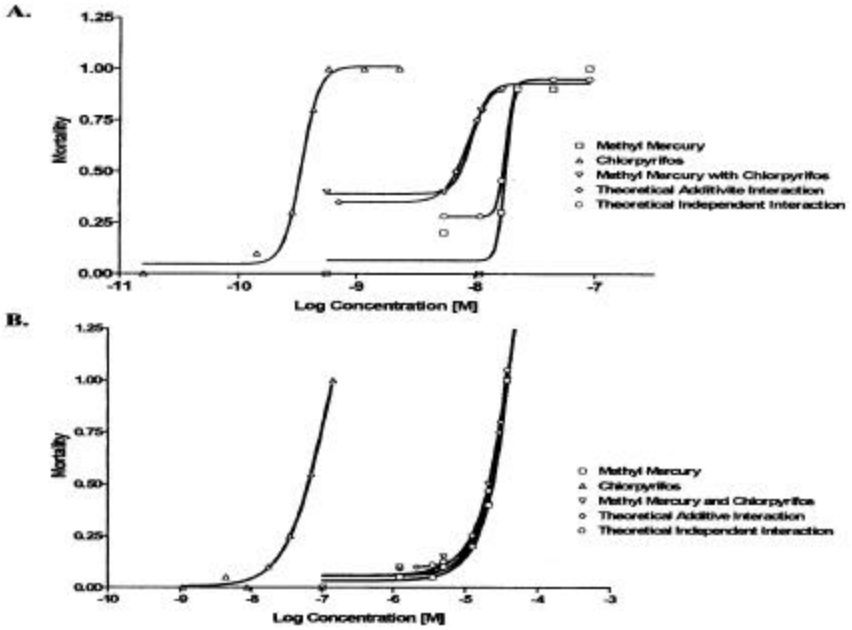

Figure 1. Survival of *Hyalella azteca* following aqueous (A) and sediment (B) exposure to individual and binary chemical mixtures of methylmercury and chlorpyrifos.

Sediment Exposures. Formulated sediments, spiked with model chemicals will be used in ten-day exposures to determine the toxicological effects of chemical mixtures at selected combinations and concentrations determined from acute toxicity tests. Formulated sediments will be utilized to provide a consistent homogenous material representative of freshwater sediment. Sediments will be formulated using Mystic White #18 sand, ASP 400 silt, and ASP 600 and 900 clay, milled humus and dolomite (Suedel and Rodgers, 1994). Components of the sediment will be aged in flowing water for seven days prior to spiking with model chemicals. Guidelines developed by ASTM (1994) will be utilized for sediment spiking. *H. azteca* will exposed to spiked sediment for ten-days according to methods described by Steevens et al. (1998) and outlined by the U.S. EPA (1994b). Sediment chambers will consist of 300 mL lipless glass beakers containing 100 mL of spiked formulated test sediment, 175 mL of overlying water, and ten juvenile test organisms. Overlying water in the sediment chambers will be renewed every 12 hours using a Zumwalt water splitting renewal system (Zumwalt *et al.*, 1994). Organisms will be fed a mixture of 1.8 mg/L yeast, cerophyll, and trout chow daily. Temperature, dissolved oxygen, and pH will be monitored throughout the ten-day exposure period. Water quality parameters including hardness, alkalinity, conductivity, and ammonia are measured at the initiation and completion of the experiment. At termination of the exposure, surviving *H. azteca* will be recovered from the sediment by sieving with a No. 50 U.S. standard sieve and preserved in 8% sugar formalin for length measurements (Tomasovic *et al.*, 1995).

Bioaccumulation and Critical Body Residues. Bioaccumulation is the accumulation in an organism's whole body tissue of a chemical directly from the aquatic environment. The bioconcentration factor is a term describing the bioconcentration of a chemical when an organism is exposed via water. Toxicity data and the bioconcentration factor will be utilized to further characterize the dose-effect relationship for a chemical. The critical body residue is the chemical concentration measured in an organism's whole body that is associated with a measured adverse toxicological effect. It utilizes a one-compartment model to determine the total dose of a chemical that the organism receives from exposure via water, food, or sediment. The critical body residue model accounts for variability in bioavailability of the chemical in the exposure media, metabolism, as well as uptake and depuration kinetics. By measuring tissue residue from field collected organisms and comparing those levels to critical body residues that we calculate from controlled experiments our proposed study will more accurately assess risk to aquatic organism during periods of vector control application.

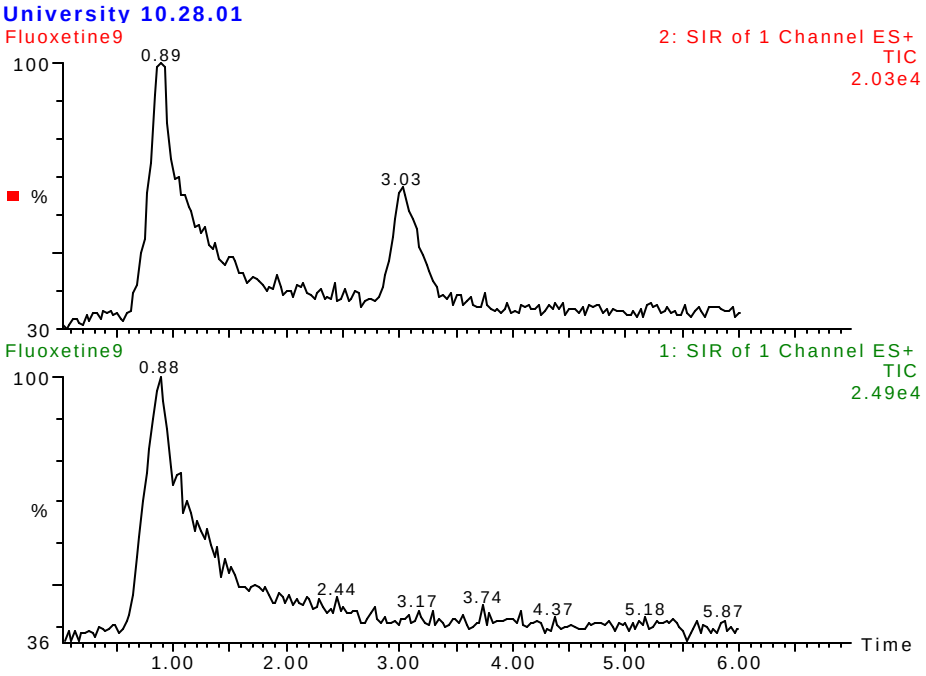

Figure 2. Chromatographs from University, MS wastewater effluent. Top chromatograph is fluoxetine and the bottom is norfluoxetine.

Chemical Analyses. Chlorpyrifos will be analyzed using an enzyme linked immunosorbant assay developed by Ohmicron Rapid Assay® (Strategic Diagnostics). Antibodies raised specifically to chlorpyrifos conjugated to magnetic particles are used to detect analyte concentrations in water and tissue samples. Tissue is prepared and analyzed by methods described by Allgood *et al.* (1997). Analytes are verified by chemical analysis using gas chromatography. Total mercury and methyl mercury is determined by solvent extraction and detected using a Varian Spectra AA-20 atomic absorption spectrophotometer and VGA-76 vapor generation system (Filippelli, 1987). The WNV vector eradication insecticide analysis will be

conducted using an LC-MS method that we are currently developing (see Weston, Huggett, Foran, Rimoldi & Slattery 2001 for example, Figure 2).

Experimental Design and Data Analysis. A mixture design will be utilized to characterize the toxic effects of mixtures using survival and growth as toxicological endpoints (Benson, Block, Steevens, Allgood & Slattery 2000). Utilizing preliminary results from single chemical toxicity data, mixture designs are ideal for studying chemical interactions (Svendsgaard and Hertzberg, 1994). The design incorporates five combinations tested at selected concentrations to provide a comprehensive analysis of the potential chemical interactions. Concentrations selected for the mixture design will be based on single chemical toxicity range finding experiments. Chemical mixture interactions based on our experimental design will be plotted using the two-sided isobolographic method. Analysis for interactions deviating from additivity will be determined using three methods including the joint action ratio, combination index, and best-fit regression analysis. The joint action ratio will be used to characterize the binary chemical interactions (Hewlett, 1969). A ratio of the magnitude of a diagonal line drawn from the origin to the observed isobole and predicted additivity describes the type of interaction. Ratios of greater than one describe synergism, and ratios of less than one describe antagonism. The combination index Ic is a mathematical model developed by Berenbaum (1989). The model is the basis for the hazard index that the U.S. EPA presently utilizes to evaluate chemical mixtures (U.S. EPA, 1989). The components of the model (d) is the effect concentration of chemical in the mixture, where (D) is the effect concentration of the chemical alone. Values for the combination index indicate additivity if Ic is equal to one, synergy if Ic is less than one, and antagonism if Ic is greater than one. Best-fit regression analysis will be used to determine the linearity of resulting data as compared to the estimated line of additivity (Gessner, 1995). Data from whole organism responses such as survival and growth as well as biochemical mechanistic data will be evaluated using the described procedure. In the case of single point interaction data, Student's t-test and analysis of variance (ANOVA) will be used. SigmaStat/Plot statistical analysis and graphing software will be utilized (Jandel Scientific).

Facilities

The facilities in the School of Pharmacy's Environmental Toxicology Research Program at The University of Mississippi that are currently available for this investigation can be divided into four major areas: (1) laboratories for basic toxicological research, (2) a Pharmacogenetics Core Facility, (3) an Aquatic Toxicology Laboratory and (4) an Environmental Toxicology Analytical Laboratory.

Basic laboratories are equipped with analytical and microbalances, scintillation counter, centrifuges, refrigerators, water baths, and an ultra-cold freezer. In addition, microscopes (Olympus B-Max 40; Olympus MEIJI), a cryostat (Leica CM1850), a rotary microtome (Olympus HM 315), and paraffin embedding station (Reichert-Jung Histembedder) are available for histological examination of tissues. A digital image analyzer system (Kodak Catseye DKC-5000 with Image Pro Plus version 3.03 software) is available for histological analysis and quantifying the size of adult, larvae, and eggs of aquatic vertebrate and invertebrate species. A TECAN SLT Rainbow UV-VIS scanning microplate spectrophotometer with WinSelect version 2.0 software is utilized for biochemical measurements. Field analysis of water quality is performed with a Hydrolab Quanta water quality monitoring system. There are several desktop

and notebook computers available for word processing and data handling and analysis. Recently, the PI equipped these laboratories with an Agilent GC/MS , a Waters LC/MS, and a JOEL SEM to provide greater toxicological identification abilities.

The Pharmacogenetic Core Facility located within ETRP's suite of laboratories has recently been outfitted with state of the art molecular analysis equipment. At the heart of the facility are a Beckman Coulter CEQ 8000 Genetic Analysis System, an Agilent 2100 Bioanalyzer and a BioRad VersaDoc 3000 image analyzer. A technician is on staff to run samples. High quality water is provided by a Millipore Milli-Q system.

The Aquatic Toxicology Laboratory is equipped for specialized research with aquatic invertebrate and vertebrate species. The Laboratory is made up of nine rooms that have individual temperature and lighting controls and Gast Regenair Blowers to provide tank aeration. Ultra pure water is supplied by a Barnstead NANOpure Infinity system. Dechlorinated water is provided by Model 2952 organic bed service exchange carbon for chlorine and chloramine removal (U.S. Filter Systems). Individual Model 2952 systems have been installed in each wet lab. There are numerous exposure systems (30- and 80-L aquaria and Frigid Unit Living Streams). For incubation of eggs, Precision Refrigerated Dual-Program Illuminated Incubators are available.

The Environmental Toxicology Analytical Laboratory occupies approximately 2,000 square feet within a 8,000 sq. ft. facility. Analytical equipment consists of a Hewlett-Packard Model 8452A diode array UV-VIS spectrophotometer with auto-sampler and kinetics software, two Hewlett-Packard Model 5890 Series II gas chromatographs (GCs) with dual electron-capture detectors, a Hewlett-Packard Model 5890 Series II GC with flame photometric and flame ionization detectors, a Hewlett-Packard Model 6890 GC with flame ionization and nitrogen-phosphorous detectors. The GCs are linked with a Hewlett-Packard Vectra 25 GC data station with Hewlett Packard Chemstation software. Also included is a Waters Model 600E HPLC system with Model 484 UV Absorbance Detector, Model 717 autosampler, a fraction collector and Millenium 2010 chromatography software. The laboratory is also equipped with an Ohmicron RPA1 Analyzer for analysis of chemicals using enzyme linked immunosorbent assays. For analysis of metals, a CEM Model MDS-2100 Microwave Digestion System as well as Varian SpectrAA-20 and SpectrAA 400 Zeeman atomic absorption spectrometers are available. A Brucker BioApex 30es High Resolution Fourier Transform Mass Spectrometer is maintained in the School of Pharmacy and is available for use in this project. Through the 1997 National Research Council of Canada/National Oceanic and Atmospheric Administration Intercomparison Studies (NOAA/10) the analytical laboratory has earned a rating of Very Good for accuracy evaluation of sediments and Superior for accuracy evaluation of biological tissues.

(13)Related Research:

Chemical Mixture Toxicity. Chemicals in the environment rarely occur alone, however, most toxicological studies are conducted using single chemical exposures. Therefore, it is necessary to characterize the toxicological hazards and risks associated with multiple chemical exposures (Parrott and Sprague, 1993; Feron *et al*, 1995). Chemicals occurring in complex mixtures have the potential for chemical-to-chemical, toxicokinetic and toxicodynamic, interactions affecting the resulting toxicological response. Chemical mixtures are characterized as having additive, synergistic, or antagonistic interactions and effects on the measured toxicological endpoint

(Calabrese, 1991). Additivity is the summation of toxic responses from multiple chemicals in a mixture. Synergism is the interaction of multiple chemicals in which the toxic response is greater than would be predicted by simple summation. Antagonism is the interaction in which the toxic response is less than would be predicted by summation. The deviation of chemical mixture toxicity from traditional individual toxicological testing makes it necessary to evaluate mixture interactions further so that the hazards and risks associated with multiple chemical exposure may be assessed (Sexton *et al.*, 1995).

To date, aquatic toxicology studies have typically evaluated the interaction of chemicals having similar mechanisms of toxicity. Kraak *et al.* (1994) studied the effects of a mixture of cadmium, copper, and zinc in the Zebra Mussel (*Dreissena polymorpha*) and determined the mixtures to be additive. Similarly, zinc and copper were found to interact additively in the Rainbow Trout (Lloyd, 1961). Spehar and Fiandt (1986) observed mixtures of metals at concentrations acceptable by the individual water quality criteria were not protective of daphnids and fish due to additivity interaction. However, Hoagland et al. (1993) found that atrazine and bifenthrin, having dissimilar mechanisms of toxicity, were additive. Several studies in which chemicals having independent or dissimilar mechanisms of action have demonstrated non-additive interactions, and in some cases found synergistic and antagonistic effects (Marinovich *et al.*, 1996). Classical studies by Triolo and Coon (1966) demonstrated that aldrin antagonized the effects of parathion, paraoxon, as well as several other organophosphates. It is apparent that there have been a variety of conclusions drawn from chemical mixture interaction studies. Chemical interactions are more complex than the assumption of additivity presently utilized to assess the risks associated with multiple chemical contaminants in sediment. Therefore, there is a need to more fully understand the underlying mechanisms of chemical mixtures responsible for deviations from additive interactions.

Bioaccumulation. Contaminated sediments have become an increasingly important issue for human and ecological health. Presently, 15 percent of the nations lakes, 4 percent of the nation's rivers, and 100 percent of the Great Lakes have fish consumption advisories associated with them (U.S. EPA, 1996). Of the fish consumption advisories, greater than 95 percent are due to bioconcentration of chemicals including mercury, PCB's, organochlorine pesticides, and dioxin. Nationally, a reported estimate of at least 29 percent of the benthic community in fresh and marine water is impacted by contaminated sediments (Veith, 1996). Long-term exposure to contaminants in the sediment can result in bioaccumulation of the chemical contaminant reaching concentrations capable of eliciting adverse toxicological effects (Borgmann et al., 1991). Toxicity, bioaccumulation and bioconcentration data can be utilized to further characterize the dose-effect relationship of a chemical. The critical body residue is the whole body concentration in an organism associated with a measured adverse toxicological effect. It accounts for variability in chemical bioavailability in the exposure media, metabolism, and uptake and depuration kinetics. The use of critical body residues in aquatic organisms has been proposed as a method to assess sediment contamination and the potential toxicological effects in aquatic organisms. McCarty and Mackay (1993) suggested the use of critical body residues and corresponding biological responses be studied to validate laboratory and field-based assessments of sediments. Currently, the assessment of sediment contamination is based on measured sediment concentrations of individual chemicals and toxicity to laboratory organisms. Safe sediment concentrations of chemical contaminants in sediment could be determined from the amount of that chemical accumulated and the corresponding measured toxicological effects.

Due to site-specific differences in chemical bioavailability and metabolism, the use of critical body residues may be a better predictor of the degree of ecological risk associated with contaminated sediments than sediment concentrations alone (Landrum *et al.*, 1992; Borgmann *et al.*, 1993).

Electronic databases used to review the literature discussed above include: Environmental Sciences and Pollution Management Abstracts (Cambridge Scientific), Life Sciences Periodical Abstracts (Cambridge Scientific), Biological and Agricultural Index (H.W. Wilson), and Biological Abstracts Inc.

Progress on Work to Date

The current proposal builds upon our previous WRRI work on chemical mixtures (Benson & Slattery: GR-02679-18) and the data we have generated on interactive effects. Specifically we tested three model compounds, chlorpyrifos, dieldrin, and methyl mercury as single- and binary-exposures on *Hyalella azteca*. We evaluated mortality, accumulation, and growth following exposure to the three model compounds, and then assessed a number of biochemical endpoints. Based on this work (and see Benson, Block, Steevens, Allgood, Slattery 2000) we noted that dieldrin acts independently in chemical mixture toxicity assays, while the chlorpyrifos:methyl mercury interaction results in significantly different effects that either compound alone, possibly due to the formation of an unknown complex. Reproductive toxicities and bioaccumulation were also significantly enhanced in the presence of mixtures. Thus, for the purposes of the present study we will focus on the chlorpyrifos:methyl mercury mixture, and examine the effects of this stressor on ability to cope with the relevant WNV vector eradication compounds (Table 2).

We have also initiated work on LC-MS method development for isolation of relevant anthropogenic contaminants in aquatic systems (Figure 1). To date this work, the focus of Mr. Weston's Ph.D., has examined the SSRI's Fluoxetine and Norfluoxetine (Weston, Huggett, Rimoldi, Foran Slattery 2001, and see below). We plan to similarly develop LC-MS techniques for the relevant WNV vector eradication compounds (Table 2), such that we can monitor fate <u>in situ</u>.

(14) <u>Investigator's qualifications:</u>

Marc Slattery is an Associate Professor of Pharmacognosy and a member of the Environmental Toxicology Research Program within the School of Pharmacy at the University of Mississippi. Dr. Slattery attended Loyola Marymount University where he obtained a Bachelor of Science degree in Biology (Marine and Environmental Biology Option) in 1981. He obtained his M.A. in Marine Biology from San Jose State University at the Moss Landing Marine Laboratories in 1987 and then worked for 3 yrs in the San Francisco Bay Area as an environmental consultant of waste-water discharge and marine toxicology. Dr. Slattery obtained his Ph.D. in Chemical Ecology from the University of Alabama at Birmingham in 1994. He has published over 35 scientific publications and has been involved in approximately $1.5 million in grants and contracts dealing with environmental toxicology and chemical ecology. His research activities have focused on assessing the acute and chronic health effects of environmental contaminants including metals, pesticides and endocrine disrupters. He has also

examined the direct and indirect effects of natural and anthropogenic stresses on reproduction and disease resistance. See attached resume for additional details.

(15) Training potential:

ESTIMATED STUDENTS RECEIVING TRAINING

Currently a single Ph.D. graduate student (Biology- Environmental Toxicology emphasis) has been targeted for salary support and training under this WRRI program. Jim Weston has been a research scientist in our Environmental Signals & Sensors research program, and he recently decided to return to grad school to complete a Ph.D. This grant will provide the support for him to complete this degree, while performing work closely related to his own interests (the environmental consequences of mixtures of pharmaceuticals in aquatic systems). However our work, and techniques, is multi-disciplinary and it is likely that several other graduate students in my lab and within the ETRP and Biology programs will assist, and be

trained, in various aspects of the project. My graduate students include Ph.D. (2 in Pharmacognosy) and M.S. (1 in Biology) candidates, and I currently fund a 4th yr undergraduate as a laboratory technician in environmental toxicology. In addition, environmental toxicology collaborations with Kristie Willett (Pharmacology/ETRP), John Rimoldi (Medicinal Chemistry/ETRP) and Stephen Threlkeld (Biology) suggests that some of their students will also be involved in either field or laboratory-based training associated with this project.

INFORMATION TRANSFER PLAN

A critical issue that has been overlooked in the recent WNV eradication discussions is the impact of spraying on environmental health. While all of the proposed mosquito control agents have been tested utilizing standard EPA protocols, *these have largely focused on single chemical dosing regimes and aquatic systems typically are comprised of chemical mixtures.* These mixtures have the potential to work additively or synergistically, and the stress of exposure to one class of compound may exacerbate the effects of another compound, even if it is applied only transiently. **Thus our goal is to assess the effects of WNV vector eradication agents in two model populations of aquatic invertebrates under conditions of single chemical doses following exposure to a mixture of persistent pesticides.**

This research program targets several important user groups: 1) the health of Mississippi residents who fish our waterways for subsistence or recreation is potentially impacted by bioaccumulation of pesticides and metals, 2) several commercial fishery markets in Mississippi (most notably Crayfish) have the potential to be either directly or indirectly impacted by mosquito adulticides and larvacides, and 3) it goes without saying that the recreation and/or tourism potential of Mississippi aquatic systems might be adversely impacted by changes in environmental health.

Our strategy for dissemination of our data will follow two closely allied approaches. First we intend to provide our results to the scientific community via presentations (budgeted regional & national mtgs) and publications in as timely a manner as possible. We also believe it is important to open a forum for discussion of problem with the lay public and the regional health councils who are developing these eradication plans. We intend to give seminars to regional user groups and develop a link/listserv to the UM ETRP page focused on this issue.

We expect to reach our target audiences via existing collaborations between ETRP and the Field Station Extension Service. And through announcements provided to the WRRI (i.e., LORE newsletter, etc).

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ABSTRACT

Project Title: Chemical Mixtures: Consequences of WNV Eradication on Water Quality

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Technical Abstract

Recent outbreaks of West Nile Virus (WNV) throughout the United States, and particularly in the Mississippi Valley States, have spurred plans for vector (= *Culex* mosquito) eradication using a variety of control insecticides. Via direct or indirect routes these compounds enter the aquatic environment where they become part of water and sediment matrices. Through direct contact, respiration or indirect ingestion non-target organisms are exposed to persistent and transient anthropogenic compounds and their mixtures. Individually or as mixtures, acting additively or synergistically, these compounds can directly affect adult and juvenile life stages of aquatic organisms. Besides direct effects some anthropogenic compounds found in aquatic matrices are known to bioaccumulate and biomagnify. At the present time, there is limited knowledge regarding effects of WNV vector control compounds in mixtures. Evaluating water quality and aquatic habitat are critical to an overall assessment of vector eradication programs.

The overall goal of the proposed research is to evaluate "real-world" chemical mixtures with toxicological effects not predicted from single chemical toxicity experiments. The amphipod *Hyalella azteca* and the water flea *Daphnia magna* will be exposed to mixtures of three model compounds: methoprene, a mosquitocide, and two regionally persistent anthropogenic compounds, chlorpyrifos and methylmercury. Bioconcentration data along with toxicological indices will be used to determine the critical body residue threshold concentrations at which toxicological effects occur. In the first year (2003-04) toxicological effects of the individual model compounds that have yet to be characterized will be assessed. In addition, we will develop analytical procedures to quantify *in situ* concentrations of model compound in regional water and sediment matrices. The second year (2004-05) will be spent toxicologically assessing mixtures of model compounds. Additional investigations will explore the impacts of pre-exposure stress on the ability of the model organisms to cope with a new toxicant. Finally, in year three (2005-06), bioconcentration data will be used to determine critical body residues. Model compounds in natural water and sediment matrices and residuals in model organisms will be quantified.